THE PROPERTIES AND SPECIFICITY OF A β-GLUCOSIDASE FROM BLABERUS CRANIIFER[1]

FRANK M. FISHER, JR.

Department of Biology, Rice University, Houston, Texas

The presence of β-glucosidase in insects was first suggested from nutritional experiments (see Lipke and Fraenkel, 1956). Since that time, the enzyme has been demonstrated in a rather large number of insect species. Such enzymatic activity has been demonstrated in crude preparations from *Porcellio* and *Armadillidium*, the pill bugs, and *Periplaneta*, the American cockroach (Newcomer, 1952, 1954, 1956). Koike (1954) investigated the β-glucosidase from *Tenebrio*, *Bombyx*, *Dictyoplaca* and *Epicauta;* and by the use of fluorometric methods, Robinson (1956) re-examined the enzyme from *Periplaneta* and *Tenebrio* and compared it with that found in *Notonecta*, *Locusta* and *Aphis*. Evans (1956) demonstrated the enzyme in preparations from *Calliphora*, using β-phenyl glucoside as a substrate, and confirmed the report by Fraenkel (1940) that the same species could not hydrolyze cellobiose. Fraenkel (*loc. cit.*) also found that adult fly preparations could not hydrolyze α-methyl glucoside, nor were cellobiose or β-methyl glucoside utilized as food substances *in vivo*. The silverfish, *Ctenolepisma*, was shown to possess a β-glucosidase capable of hydrolyzing cellobiose (Lasker and Giese, 1956). The enzyme was also shown to be present in bacteria-free silverfish. Ito and Tanaka (1959) partially purified and characterized the β-glucosidase from *Bombyx* mid gut. Evans and Payne (1960) reported that *Schistocerca* tissue possessed the ability to hydrolyze β-linked carbohydrates. In a survey of the carbohydrases of *Blaberus discoidalis* and *Leucophaea madeirae* (*sic*), Ehrhardt and Voss (1962) described the presence of a β-glucosidase in both roach species, but no characterization was made. Recently, Powning and Irzykiewicz (1962) further investigated the β-glucosidase activity of *Periplaneta* in a comparative study in which they also included *Lycoperdon*, a puffball. The present investigation deals with the partial purification and characterization of a β-glucosidase from *Blaberus craniifer*.

Materials and Methods

Experimental material

Cockroaches were maintained in 50-pound lard tins at 24–28° C. on a diet of dog biscuits and ground Purina Lab Chow. Cedar shavings and/or crushed corn cobs were provided as litter, and fresh apples were added periodically.

[1] This work was supported in part by a National Institutes of Health, U. S. Public Health Service, Post Doctoral Fellowship and Training Grant No. 2E106.

Reagents

The buffers used in the assay procedures were made with reagent grade chemicals and glass-distilled water. The following buffers were used: (1) phthalate: NaOH, (2) citrate, (3) citrate: phosphate, (4) phosphate, (5) Tris (hydroxymethyl) aminomethane (hereinafter referred to as Tris): HCl, (6) Tris: maleate, and (7) glycine: NaOH. Substrates were also dissolved in glass-distilled water. Alpha-alpha-trehalose, β-methyl-D-glucoside, α-methyl-D-glucoside, α- and β-melibiose, p-nitrophenyl-β-D-glucoside (PNPG), o-nitrophenyl-β-D-galactopyranoside (ONPGal), and gentiobiose were obtained from California Corporation for Biochemical Research. Arbutin and amygdalin were purchased from K and K Laboratories. Cellobiose and salicin were obtained from Sigma Chemical Company, and raffinose, cellobiose, maltose and lactose from Pfanstiehl Chemical Company. Glucose oxidase ("Glucostat") was obtained from Worthington Biochemical Corporation.

Preparation of homogenates

Roaches were starved for 48 hours prior to preparation and the tissues weighed and homogenized in Tris:HCl 0.005 *M*, pH 7.4 (2:1, v/w) with a Servall Omni-Mix at 0° C. for three minutes at 14,000 rpm. The resulting homogenate was squeezed through three thicknesses of cheesecloth, rehomogenized in a Tenbroek homogenizer, and the volume measured (Fraction I). An aliquot was subjected to centrifugation at 24,000 *g* at 0° C. for 30 minutes. The supernatant was poured through glass wool to remove the fatty layer and the supernatant volume measured (Fraction II).

Purification

An aliquot of Fraction II was subjected to ethanol precipitation at −7° C., similar to the method used by Friedman (1960). Absolute ethanol was added drop-wise with constant stirring until 30% v/v was obtained, and the mixture agitated for an additional 10 minutes. The mixture was centrifuged at 24,000 *g* (0° C.) for 30 minutes, the supernatant removed and measured, and sufficient ethanol added to that supernatant at −7° C. to bring the concentration to 40% (v/v). This process was continued step-wise in 10% increments until the ethanol percentage was 70%. Each of the precipitated fractions was taken up in 5.0 ml. Tris:HCl (0.005 *M*, pH 7.4) and dialyzed against two liters of that buffer at 5° C. for 18 hours. The precipitated material from the dialyzed fractions was removed by centrifugation and the supernatant stored at −20° C. until assay.

In some preparations (isolated gut tissue), the procedure was somewhat different. Fraction II of that preparation was subjected to 29% ethanol precipitation at −7° C., and subsequent precipitations were completed on the 29% ethanol supernatant with −20° C. acetone in a refrigerated bath at −7° C. Precipitated fractions of 30%, 40%, 60%, and 80% (v/v) acetone were collected, taken up in Tris: HCl buffer (0.005 *M*, pH 7.1), dialyzed against that buffer, centrifuged as above, and stored at −20° C.

Assay procedures

Enzyme activity was normally determined as follows: 0.1 to 0.3 ml. of the enzyme solution or diluted enzyme was added to 10 μmoles (0.5 ml.) substrate and 60 μmoles (0.6 ml.) buffer. Deviations from these conditions will be discussed in the text. The mixture was incubated at 34° C. for 15 minutes and the reaction was stopped by the addition of 1.0 ml. $Ba(OH)_2$ and 1.0 ml. $ZnSO_4$, according to the method of Somogyi (1952). After centrifugation, aliquots of supernatant were removed and glucose was determined with glucose oxidase (Keston, 1956) or by the Somogyi-Nelson method (Somogyi, *loc. cit.*). When nitrophenyl glycosides were used as substrates, the mixtures were made alkaline by the addition of 0.1 *N* $NaCO_3$ and read directly at 400 mμ. Standard glucose curves were established by adding the equivalent volume of the particular disaccharide or glycoside substrate to each individual concentration of glucose and the water blank. These tubes were treated with $Ba(OH)_2$ and $ZnSO_4$ in the same manner as the experimental tubes.

TABLE I

Enzymatic activity of Fraction II on various carbohydrates. Conditions: 10 μmoles substrate, 60 μmoles buffer (citrate pH 5.6), 0.1 ml. enzyme (whole animal preparation)

Substrate	μmoles/mg. protein hour
Cellobiose	1.29
Sucrose	0.98
Trehalose	0.96
Lactose	0.84
Melibiose	0.68
Raffinose	0.68
Maltose	0.54
Starch	+
Glycogen	+
Cellulose (Alphacel)	−
Methyl cellulose	−

This system afforded internally compensated control for the contaminant carbohydrases in the glucose-oxidase preparation. On some occasions, the "Glucostat" was diluted with 0.20 *M* or 0.25 *M* Tris: HCl at pH 7.1 *in lieu* of water. This addition inhibited all carbohydrates at least 95%. The glucose-oxidase reaction was conducted with 4 ml. of the reagent at 34° C. for 30 minutes. The reaction was stopped and color developed by the addition of 0.05 ml. of 6 *N* HCl and the tubes read with a #42 filter in a Klett-Summerson colorimeter. Standard curves (corrected for substrates) obtained by this method gave slopes of 0.2 (±0.02) μgm. glucose/klett unit. Transferase activity was examined according to the method of Aronson (1952). Protein was estimated by the method of Lowry *et al.* (1951) with the Folin-Ciocalteu reagent, using crystalline Armour bovine plasma albumin as standard. It was found that Sigma bovine serum albumin gave the same standard curve as did the Armour bovine plasma product. Activity of the enzymes is expressed as μmoles substrate hydrolyzed/mg. protein hour.

Paper chromatography and disc electrophoresis

Reaction mixtures were examined chromatographically, using the ascending technique on Whatman #1 paper and solvent system of ethyl acetate, pyridine,

TABLE II

Enzymatic activity of ethanolic fractionation of Fraction II. Conditions: Same as Table I

Ethanol fraction (% v/v)	μmoles/mg. protein/hour		
	Maltose	Cellobiose	Trehalose
30	0.80	1.0	0.5
40	0.80	1.25	0.90
50	0.80	2.20	1.50
60	2.50	4.00	1.40
70	6.10	0.50	0.20

methyl ethyl ketone, and water (5:4:4:1, v/v) or the descending method (Whatman #4 paper) with n-propanol, ethyl acetate, and water (7:1:2, v/v) (Barr and Bull, 1953). A modified method of the $AgNO_3$:NaOH of Trevelyan *et al.* (1950) was used to determine the carbohydrates. The chromatograms were sprayed with the acetone:$AgNO_3$ solution *in lieu* of dipping as suggested by the

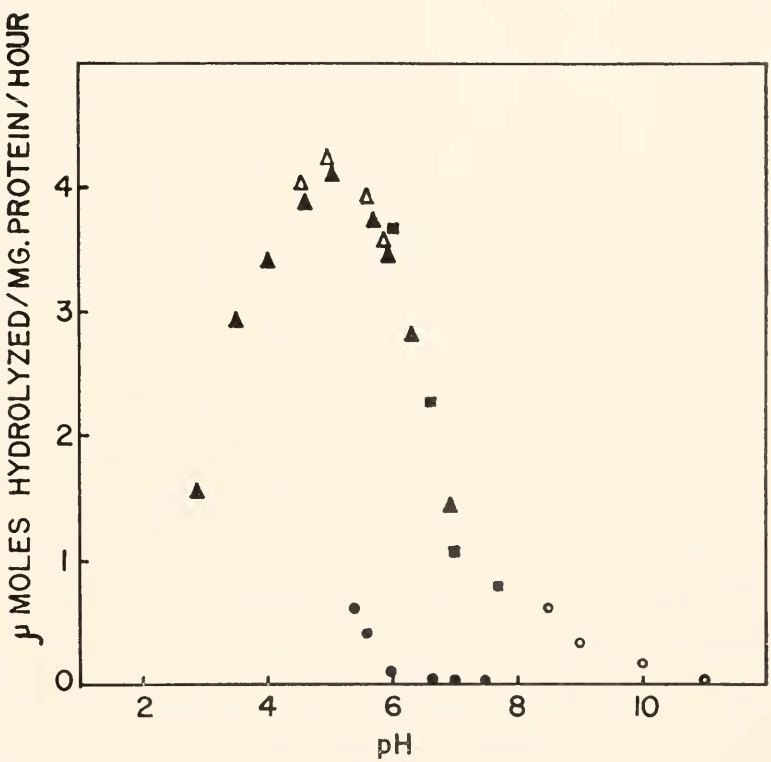

FIGURE 1. The pH optimum of *Blaberus* β-glucosidase. Conditions: 10 μmoles cellobiose, 0.1 ml. enzyme, 60 μmoles buffer. ▲-▲-citrate phosphate, ●-●-Tris:malcate, ■-■-phosphate (K^+), △-△-phthalate(Na^+), ○-○-glycine (Na^+).

authors. Chromatograms were also examined under ultraviolet light, and many of the aryl-aglycone moieties of the glucosides were visualized.

Protein fractions were examined by disc electrophoresis (Reisfeld *et al.*, 1962) on acrylamide gel incorporating Tris:glycine buffer, pH 8.3 with a potential of 200 volts. The protein bands were localized with amido black.

Results

Total Animal Preparation

Hydrolysis of carbohydrates

Assay of the supernatant of total animal homogenates (Fraction II) affords some idea of the number of carbohydrases present in *Blaberus craniifer* (Table I). In addition to those substrates shown, glycogen and starch were hydrolyzed; however, cellulose and methyl cellulose were not hydrolyzed. Precipitation of Fraction II with ethanol at −7° C. partially separated and increased the specific activity of the preparations (Table II). The α-glucosidase active against maltose precipitated largely in the 70% ethanol fraction, while the β-glucosidase activity precipitated out in the 50% and 60% fractions. Trehalase was also equally distributed in the 50% and 60% fractions.

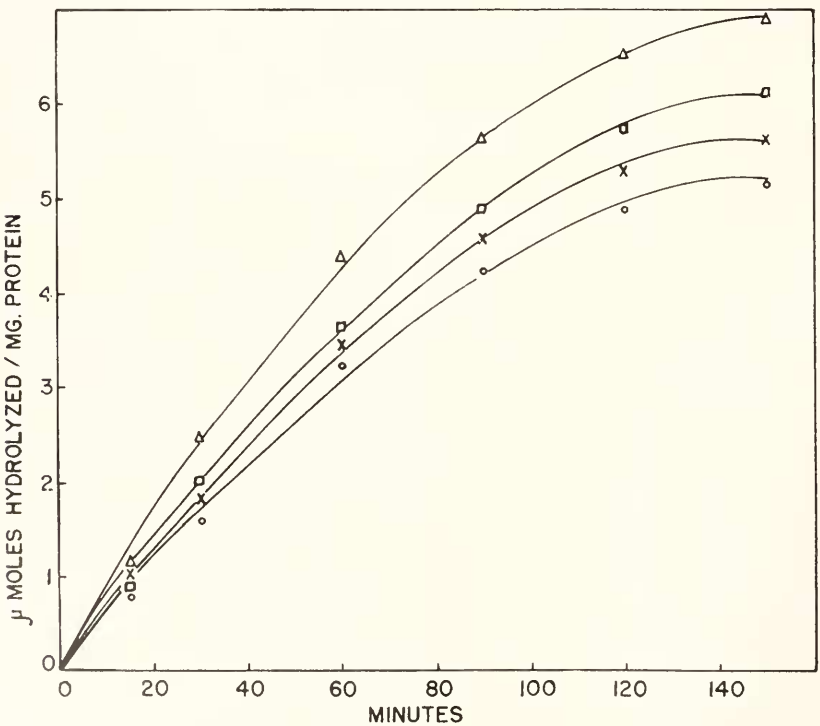

Figure 2. Effect of pH and increasing time on enzymatic activity. Phthalate (Na^+) buffers △-△ pH 5.0, □-□ pH 4.4, ×-× pH 5.6, ○-○ pH 5.8. Conditions: 10 μmoles cellobiose, 60 μmoles buffer, 0.1 ml. enzyme (40% to 60% ethanol fraction, whole animal).

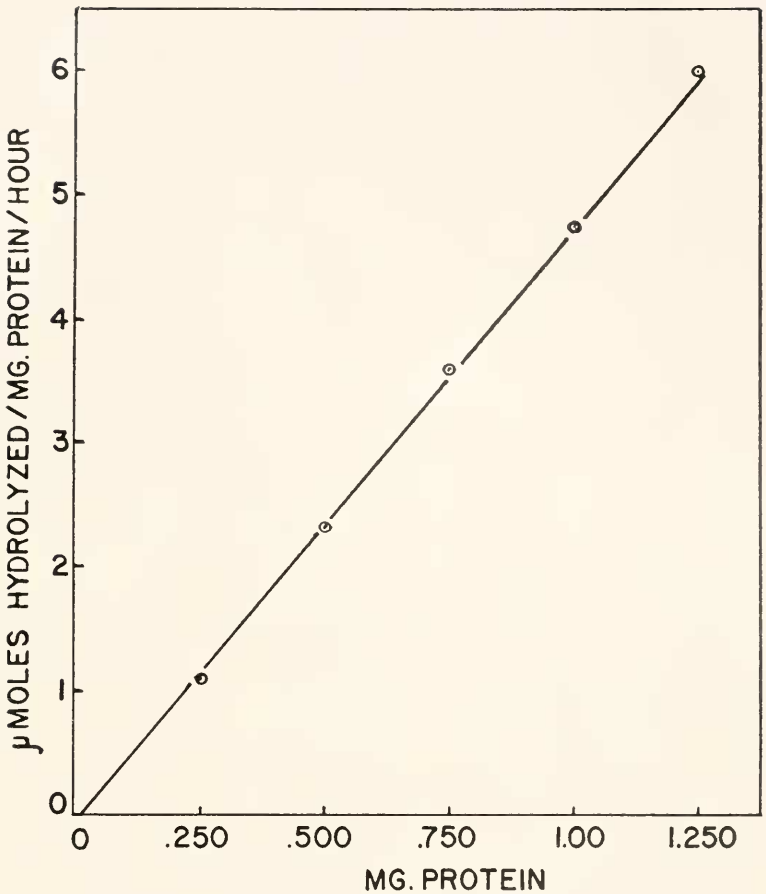

FIGURE 3. Enzymatic activity *vs.* protein concentration. Assay conditions: 10 μmoles cellobiose, 60 μmoles buffer (phthalate pH 5.0), 0.1 ml. enzyme (40% to 60% ethanol fraction, whole animal).

Effect of pH

In examining the hydrolysis of cellobiose, five buffers were used which covered a range from pH 2.9 to 11.0 (Fig. 1). The curve represents the average of four determinations at each point. At the concentration used (4.29×10^{-2} *M*), the Tris : maleate buffer inhibited cellobiose hydrolysis some 85%, while trehalose hydrolysis was inhibited only 25% under the same conditions. This is based on 100% activity in pH 5.0 phthalate buffer with which the highest activity was observed.

Effect of time and enzyme concentration

Figure 2 shows the effect of increasing time on cellobiose hydrolysis using four phthalate buffers, and is based on the average of triplicate observations. This

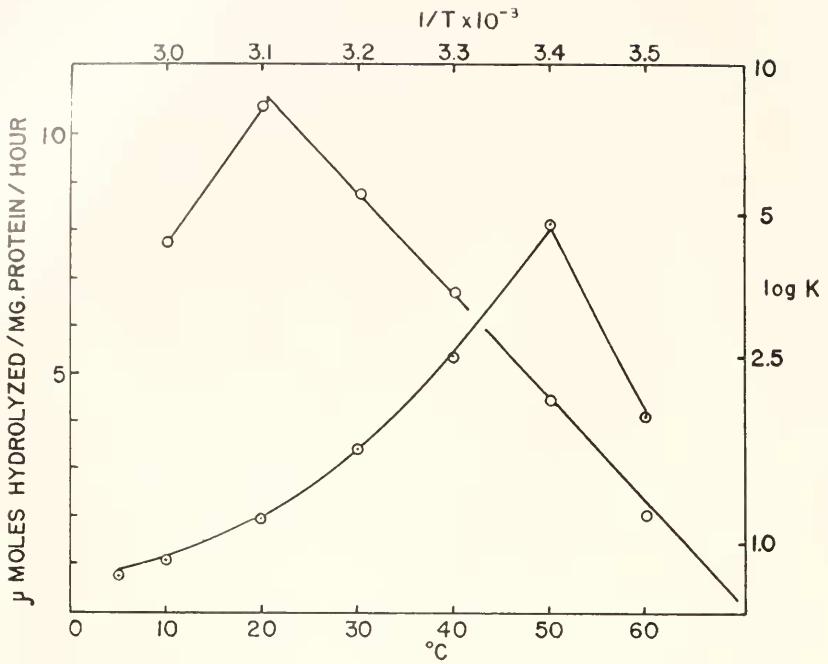

FIGURE 4. Dual plot of enzymatic activity *vs.* increasing temperature (⊙-⊙) and Arrhenius plot ($1/T$ *vs.* log K) (○-○). Conditions: 10 μmoles cellobiose, 60 μmoles buffer (phthalate pH 5.0), 0.1 ml. enzyme (40% to 60% ethanol fraction, whole animal).

TABLE III

Effect of metal ions on enzymatic activity. Conditions: 10 μmoles cellobiose, 60 μmoles buffer (phthalate pH 5.0), 0.01 ml. inhibitor, 0.1 ml. enzyme (40% to 60% ethanol fraction, whole animal)

Inhibitor ion	Final concentration (molar)	% Inhibition
None	—	0
Ag^{+}	3.00×10^{-5}	100
Ag^{+}	8.26×10^{-4}	100
Hg^{+}	8.26×10^{-4}	72
Hg^{++}	8.26×10^{-4}	65
Cu^{++}	8.26×10^{-4}	60
Pb^{++}	8.26×10^{-6}	50
Fe^{++}	8.26×10^{-4}	40
Co^{++}	8.26×10^{-4}	18
Zn^{++}	8.26×10^{-4}	16
[illegible]	8.26×10^{-4}	14
[illegible]	8.26×10^{-4}	9
[illegible]	8.26×10^{-5}	8
[illegible]	8.26×10^{-4}	4
[illegible]	8.26×10^{-4}	4
[illegible]	8.26×10^{-4}	2

figure indicates that the hydrolysis is inhibited more on the alkaline side of the peak than on the acid side. Enzymatic activity was linear as a function of protein concentration (Fig. 3), and no inhibition of activity was noted throughout the range described.

Energy of activation

The energy of activation for cellobiose hydrolysis was determined from the linear portion (5° C. to 50° C.) of an Arrhenius plot (log K *vs.* $1/T$) where the slope represents $-E/2.303\ R$. Figure 4 represents the effects of increasing temperature *vs.* activity, as well as the Arrhenius plot. From these data based on triplicate observations, the energy of activation was estimated to be 9400 calories/mole. It was further determined that 50% of the hydrolytic activity against cellobiose was lost following incubation for one hour at 50° C.

Effect of metals and salts

The inhibitory nature of Tris buffers on cellobiose hydrolysis has been mentioned previously. This is also inhibitory for a number of other glucosidases (Friedman, 1960; Mahler, 1961; White and Subers, 1961; Blecher and Glassman, 1962). Table III lists 16 metal ions and their inhibition activity, using pH 5.0 phthalate buffer as 100%. Enzyme activity was reduced by the specific sulfhydryl inhibitors, parachloromercuribenzoic acid (PCMB), phenylmercuriacetate (PMA), iodoacetate, and iodacetamide; and cysteine produced a partial reversal of these inhibitions. Iodoacetate and iodoacetamide at 2×10^{-4} M completely inhibited the enzyme, and the reversal by 4×10^{-3} M cysteine was significant (Table IV). PCMB and PMA were used at lower concentrations, and significant reversal by

TABLE IV

Effect of sulfhydryl inhibitors. Conditions: 10 μmoles cellobiose, 60 μmoles buffer (phthalate pH 5.0), 0.1 ml. enzyme (Fraction II, whole animal)

Inhibitor	Cysteine (Molar)	Concentration inhibitor (Molar)	% Inhibition
None	0	—	0
	4×10^{-3}		0
PCMB	0	2×10^{-6}	40
	4×10^{-3}	2×10^{-6}	9
	0	4×10^{-5}	62
	4×10^{-3}	4×10^{-5}	13
PMA	0	2×10^{-5}	56
	4×10^{-3}	2×10^{-5}	11
Iodoacetate	0	2×10^{-4}	100
	4×10^{-3}	2×10^{-4}	83
Iodoacetamide	0	2×10^{-4}	96
	4×10^{-3}	2×10^{-4}	79

cysteine was also observed. The effect of monovalent cations on enzymatic activity was examined, using the chloride form at a final concentration of 1.67×10^{-1} *M*. The following relationship existed:

$$K^{+} > Na^{+} > Rb^{+} > Cs > Li$$

In triplicate experiments, it was found that the overall activation of potassium was 10%, while that of lithium was less than 2%. The other cations were displaced between those points. In this series of experiments, the enhancer effect of toluene (Veibel, 1937–38) was also examined, and no increase in activity was observed.

Localization of enzyme

In assays of the total animal, isolated gut, and carcass, it was found in eight determinations that 89–95% of the β-glucosidase activity was in the gut. When the gut was divided into three portions (*i.e.*, fore-, mid-, and hindgut), it was observed that 96% of the total gut activity resided in the ceca complex. Histochemical localization of the enzyme was determined according to Pearse (1960),

TABLE V

Ethanol-acetone fractionation of gut tissue

Fraction	Volume (ml.)	Mg. protein (ml.)	Total protein (mg.)	μmoles Mg. protein/ hour	Total activity	% Recovery supernatant
Homogenate	44	29.8	1311.1	4.68	6135.9	—
Supernatant	34	20.1	683.4	6.52	4455.8	100
29% Ethanol	15.5	26.6	413.0	1.69	698.0	15.7
20% Acetone	4.2	.96	4.0	3.85	15.4	0.3
40% Acetone	4.4	1.65	7.3	3.71	27.1	0.6
60% Acetone	7.0	2.34	16.4	173.21	2840.6	63.8
80% Acetone	2.0	1.20	2.4	1.5	3.6	0.1

using 6-bromo-2-naphthol-β-glucoside as the substrate, and after coupling with fast blue B. The major enzymatic activity was found in the ceca and a small amount in the midgut tissue. The only localized area of activity in the carcass was found in the epidermis; however, the colleterial glands were not examined. The right colleterial gland has been shown in other roach species to have a β-glucosidase, and the left gland contained aryl-β-glucosides. The concomitant mixing of the secretions of these two glands resulted in the liberation of the aglycone, which was oxidized by a phenolase to a quinone. The result was a general darkening of the oötheca (Brunet and Kent, 1955; Stay and Roth, 1962).

Isolated Gut Preparation

The enzyme used in the following series was the 60% acetone fraction, prepared as described in the methods section. Table V summarizes this purification and gives the specific activities of the fractions against cellobiose. The pH optimum and the effect of metal salts were the same as obtained from the total animal preparation.

Specificity of the fraction

The specific activities of the 60% acetone fraction against 20 substrates are listed in Table VI. Five of the ten β-linked glucosides tested were hydrolyzed by this preparation. Heat treatment at 50° C. for one hour decreased the hydrolytic properties toward all of those five glucosides approximately 50%. Lactose and ONPGal were hydrolyzed, as well as trehalose and maltose. Examination of this enzyme preparation by disc electrophoresis showed two distinct small bands and a larger band which contained two components.

Table VI

Specific activities of 60% acetone fraction at pH 5.0 against various substrates. Conditions: 20 μmoles substrate, 60 μmoles buffer (phthalate pH 5.0), 0.1 ml. enzyme (60% acetone, animal gut)

β-glucosides	μmoles/mg. protein/hour
Cellobiose	173.2
Phenyl-β-D-glucoside	84.3
p-nitrophenyl-β-D-glucoside	44.1
Salicin	34.6
Arbutin	22.7
Gentiobiose	—
Amygdalin	—
β-methyl-D-glucoside	—
Cellulose (alpha-cel)	—
Methyl cellulose	—
Other carbohydrates	**μmoles/mg. protein/hour**
o-nitrophenyl-β-D-galactopyranoside	41.2
Lactose	33.1
Trehalose	28.1
Maltose	15.8
β-melibiose	—
Melibiose	—
Sucrose	—
Raffinose	—
Starch	—
Glycogen	—

Transferase activity

No transferase activity was observed when methanol, ethanol, glucose, hydroquinone and p-nitrophenol were used as acceptors and cellobiose as the donor. Mixtures fortified with adenosine triphosphate (ATP) and/or uridine-diphosphoglucose (UDPG) were negative for transferase and/or uridine-diphosphate-glucose glycosyltransferase activity. Reaction mixtures were examined chromatographically, and only increasing amounts of glucose and decreasing amounts of cellobiose were observed in reaction mixtures incubated 0.25, 0.5, 1, 3, 6, 18, 24, and 120 hours. Dutton (1962) has reported that intact and homogenized cecum and fat body of *Periplaneta* bring about the conjugation of o-aminophenol only in the presence of UDPG.

Effect of substrate concentration and inhibition

The effect of cellobiose concentration *vs.* enzymatic activity was examined using nine substrate concentrations over a 100-fold range. The K_m was estimated to be 5.63×10^{-4} *M* and the V_{max} 1.89×10^{2} μmoles/mg. protein hour from the double

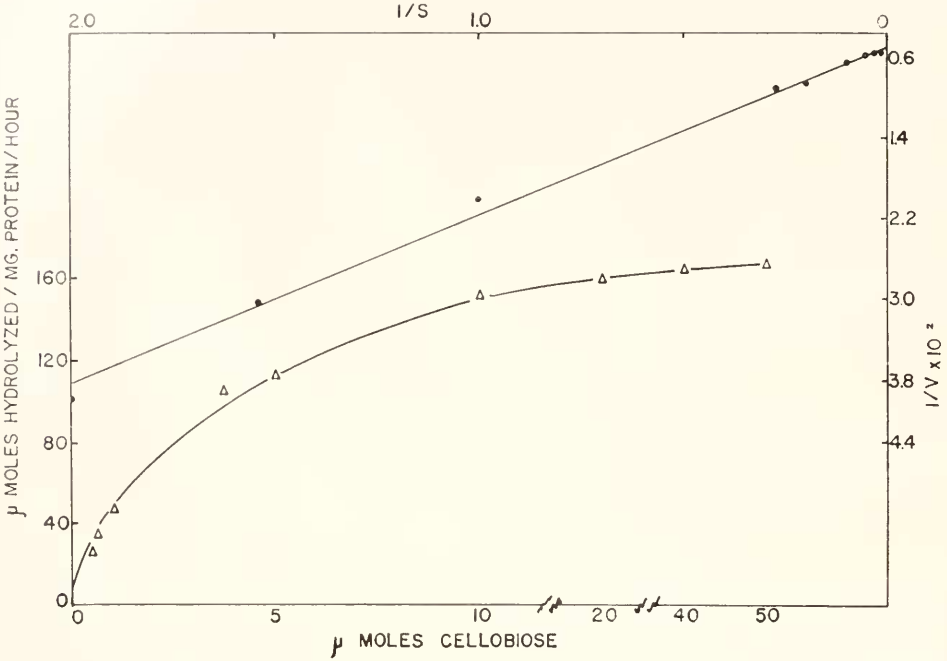

FIGURE 5. Substrate concentration plot: (1) △-△ specific activity *vs.* concentration of cellobiose; (2) ●-● $1/v$ *vs.* $1/s$. Conditions: 60 μmoles buffer (phthalate pH 5.0), HOH + substrate to equal 1.7 ml., 0.1 ml. enzyme (60% acetone fraction, animal gut).

TABLE VII

K_m and K_{max} values against various glucosides. Conditions: 20 μmoles substrate, 60 μmoles buffer (phthalate pH 5.0), 0.1 ml. enzyme (60% acetone fraction, animal gut)

Substrate	Inhibitor	$K_m \times 10^3$	Apparent $K_m \times 10^3$	V_{max}	$K_i \times 10^3$
p-nitrophenol β-D-glucoside	—	16.56		62.5	—
	Lactose 2.5×10^{-2}		56.17	55.6	9.0
	Cellobiose 2.5×10^{-2} *M*		126.40	58.8	3.0
	Glucose 1.25×10^{-2} *M*		65.07	58.1	4.0
o-nitrophenol-β-D-galactopyranoside	—	389.1		76.9	—
	Lactose 2.5×10^{-2} *M*		426.8	77.9	303.0
	Cellobiose 2.5×10^{-2} *M*		847.8	66.7	15.0
	Glucose 1.25×10^{-2} *M*		410.2	83.3	136.0
Phenyl-β-D-glucoside		23.2		14.3	
Arbutin		1.2		14.7	
Salicin		0.621		17.2	
Cellobiose		0.563		188.6	

reciprocal plot (Fig. 5). The K_m and V_{max} values for other β-glucosides and ONPGal are presented in Table VII. Also given in that figure are the results obtained when PNPG and ONPGal were used as substrates and lactose, cellobiose and glucose as inhibitors.

Further purification of 60% acetone fraction

A portion of the 60% acetone fraction was dialyzed against 0.005 *M* Tris: maleate, pH 8.4, and placed on a 1 × 15 cm. DEAE cellulose column prepared according to Peterson and Sober (1956) and a gradient elution effected with a two-container closed system under 1.5 psi nitrogen gas at 5° C. The mixing chamber contained 200 ml. Tris:maleate 0.005 *M*, pH 8.4, and the flask delivering

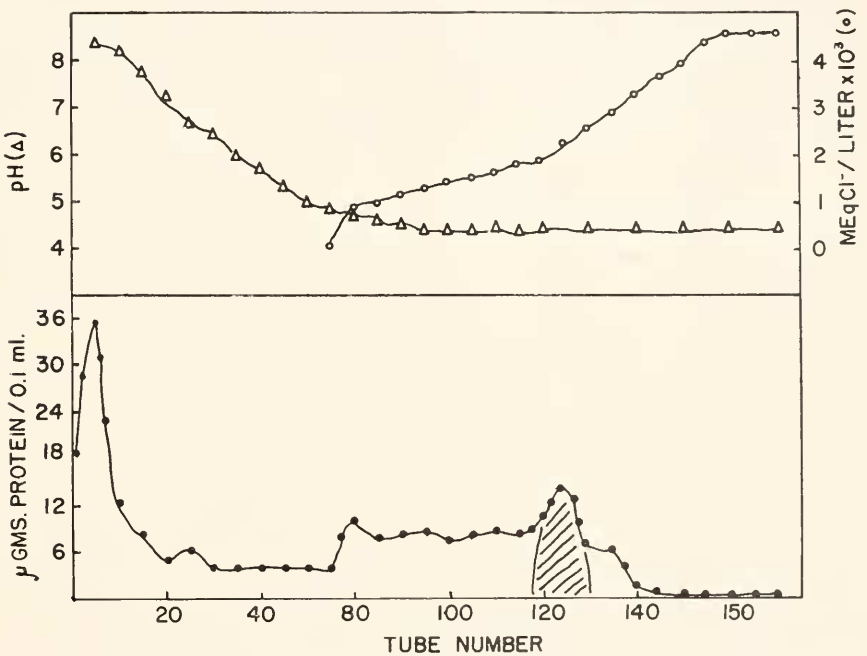

FIGURE 6. Elution patterns from DEAE cellulose column. The pH curve is designated by: △-△, Cl (meq/l.) by ○-○; and protein by ●-●, shaded area = enzymes.

to the mixing chamber contained 700 ml. of the same buffer at pH 4.6. After the above elution was complete, the mixing chamber was charged with 500 ml. Tris: maleate 0.005 *M*, pH 4.6, and 200 ml. 0.5 *N* NaCl was added to the delivery flask. When 100 ml. ± 25 ml. remained in the mixing chamber, 200 ml. of 2.0 *N* NaCl were added to the delivery flask and elution continued. Fifteen-milliliter-samples were collected throughout the elution. The profile of protein eluted from the column is shown in Figure 6. The pH gradient is presented, as well as the Cl^- concentration as meq/l. Chloride was determined with a Cotlove chloridometer and pH with a Beckman probe-type electrode. Beta-glucosidase activity is indicated by the hatched area. Beta-glucosidase activity and a trace of β-galactosidase

activity were observed in the peak. Lyophilization of the protein from the enzyme peak after preliminary assays destroyed about 96% of the activity. Alpha-glucosidase and trehalase activities were not in the indicated peak of enzyme activity.

Discussion

Protein preparations from *Blaberus* hydrolyze numerous carbohydrates, and these enzymatic activities may be partially separated by ethanol precipitation at $-7°$ C. (Figs. 1 and 2). The major source of hydrolytic enzymes is the intestine; however, the extra-intestinal tissues possess at least trehalase and a β-glucosidase. Maximum β-glucosidase activity is found at pH 5.0 (Fig. 3). The effect of increasing time on the hydrolysis of cellobiose in four phthalate buffers shows that maximum hydrolysis in 150 minutes occurs at pH 5.0; 89% of that activity was observed at pH 4.4, 81% at pH 5.6, and 74% at pH 5.8 (Fig. 4). The rate of hydrolysis was little affected by the addition of Na^+, K^+, Li^+, Rb^+, Cs^+ and Cu^+; however, Ag^+ and Hg^+ had a pronounced inhibitory effect. Among the divalent ions (Fig. 7), Sn^{++}, Mn^{++} and Mg^{++} were without appreciable effect, while Hg^{++}, Cu^{++}, Pb^{++}, Co^{++}, Zn^{++} and Fe^{++} inhibited hydrolysis in excess of 14%. Of the trivalent metals tested, Fe^{+++} was more effective than Al^{+++}. Similar results have been reported for β-glucosidases of almond (Veibel, 1951); and for β-glucosidase (Duerksen and Halvorson, 1958), α-glucosidase (Halvorson and Ellias, 1958), and invertase of yeast (Myrback, 1957). The effect of these metal ions suggested the presence of sulfhydryl groups ($-SH$); and the inhibitory effect of $-SH$ inhibitors was examined, as well as the reversal effect of cysteine. Iodoacetate, iodoacetamide, PCMB and PMA all provided inhibition of hydrolysis of cellobiose; the inhibition was partially reversed by the addition of 4×10^{-3} *M* cysteine (Fig. 8). No studies were undertaken which would elucidate the involvement of these groups in hydrolytic activity, as has been shown by competitive substrate protection experiments with yeast β-glucosidase (Duerksen and Halvorson, 1958).

By treatment of the ethanolic supernatant fraction (29% v/v) of the roach gut preparation with increasing percentages of acetone (v/v), the specific activity was increased some 37-fold (Fig. 9). The 60% acetone fraction from this purification was tested against a number of carbohydrates (Fig. 10). Of the β-linked carbohydrates examined, cellobiose, phenyl-β-D-glucoside, PNPG, salicin and arbutin were hydrolyzed. Amygdalin, gentiobiose, β-methyl-D-glucoside, cellulose and methyl cellulose were not hydrolyzed. These data suggest that the $\beta 1 \rightarrow 6$ linkage of gentiobiose and the glycone of amygdalin, which is also gentiobiose, cannot be hydrolyzed by this enzyme. The fact that cellulose cannot be hydrolyzed indicates that no exoglucosidase activity is present. Also, methyl cellulose and β-methyl-D-glucoside are not hydrolyzed; and this suggests that there is some specificity toward the aglycone moiety of the glucoside. Similar results have been discussed by Jermyn (1961).

It is of interest that the partial inactivation of the enzyme by heat results in the proportional loss (*ca.* 50%) of activity toward all of the five β-glucosides hydrolyzed. This favors the notion that each of these glucosides is hydrolyzed by a common site, as reported by Weidenhagen (1932). Under similar treatment, lactose, maltose and trehalose hydrolysis was decreased 40%, 62% and 59%, respectively.

The 60% acetone fraction was further examined by using aryl substrates with cellobiose, glucose, and lactose as inhibitors. Cellobiose and glucose were competitive inhibitors of PNPG, and lactose was competitive against ONPGal. On comparing the K_i values (Fig. 12) which were determined from the calculated slopes, it is obvious that two enzymes or two active centers are present, one for β-glucosides and another for β-galactosides. This is not consistent with the notion that these two classes of compounds are hydrolyzed at the same enzyme site, as reported for almond emulsion by Heyworth and Walker (1961).

The author wishes to thank Mrs. Marillyn Gragg and Mr. Robert McRitchie for technical assistance. Thanks are also extended to Dr. C. P. Read for his comments and criticisms and to Dr. R. C. Sanborn for the loan of equipment.

Summary

1. The localization, properties and specificity of a β-glucosidase from *Blaberus craniifer* have been investigated.

2. The enzyme is localized mainly in the cecal complex of the alimentary canal.

3. Evidence is presented which suggests that all the β-glucosides hydrolyzed are hydrolyzed by the same enzyme and that the β-galactosides are hydrolyzed by another protein or series of active sites.

4. The data also suggest that some specificity of the enzyme is directed toward the aglycone moiety of the β-glucoside.

5. Based on inhibition of the enzyme by heavy metals and specific $-SH$ inhibitors and the reversal of $-SH$ inhibition by cysteine, it is suggested the enzyme is an $-SH$ enzyme.

6. No effects of toluene were noted and the effects of monovalent cations were less than 10%.

7. The K_m, V_{max} and K_i values for various glucosides are presented.

LITERATURE CITED

Aronson, M., 1952. Transgalactosidation during lactose hydrolysis. *Arch. Biochem. Biophys.*, **39**: 370–378.

Barr, S., and J. P. Bull, 1953. Salt interference in sugar chromatography of urine. *Nature*, **172**: 414–415.

Blecher, M., and A. B. Glassman, 1962. Determination of glucose in the presence of sucrose using glucose oxidase; effect of pH on absorption spectrum of oxidized o-dianisidine. *Analyt. Biochem.*, **3**: 343–351.

Brunet, P. C., and P. W. Kent, 1955. Mechanism of sclerotin formation—the participation of a beta-glucosidase. *Nature*, **175**: 819–820.

Duerksen, J. D., and H. Halvorson, 1958. Purification and properties of an inducible β-glucosidase of yeast. *J. Biol. Chem.*, **233**: 1113–1120.

Dutton, G. J., 1962. The mechanism of o-aminophenyl glucoside formation in *Periplaneta americana*. *J. Comp. Biochem. Physiol.*, **7**: 39–46.

Ehrhardt, P., and G. Voss, 1962. Beitrag zum Wirkungsspektrum Kohlenhydratspaltender Fermente und ihre Verteilung im Verdauungstrakt der Schaben *Blaberus discoidalis* Sv. und *Leucophaea madeirae* [sic] F. (Blattoidea). *J. Ins. Physiol.*, **8**: 165–174.

Evans, W. A. L., 1956. Studies on the digestive enzymes of the blowfly *Calliphora erythrocephala*. I. The carbohydrases. *Exp. Parasitol.*, **5**: 191–206.

Evans, W. A. L., and D. W. Payne, 1960. Carbohydrases in the alimentary tract of the locust *Schistocerca gregaria* (Forsk.). *Biochem. J.*, **76**: 50.

Fraenkel, G., 1940. Utilization and digestion of carbohydrate by the adult blowfly *Calliphora erythrocephala*. *J. Exp. Biol.*, **17**: 18–29.

Friedman, S., 1960. The purification and properties of trehalase from *Phormia regina* Meig. *Arch. Biochem. Biophys.*, **87**: 252–258.

Halvorson, H., and L. Ellias, 1958. The purification and properties of an α-glucosidase from *Saccharomyces italicus* Y 1225. *Biochim. Biophys. Acta*, **30**: 28–40.

Heyworth, R., and P. G. Walker, 1961. Identity of β-glucosidase and β-galactosidase in sweet-almond emulsin. *Biochem. J.*, **80**: 44.

Ito, T., and M. Tanaka, 1959. Beta-glucosidase of the mid gut of the silkworm *Bombyx mori*. *Biol. Bull.*, **116**: 95–105.

Jermyn, M. A., 1961. Glycosidases. *Revs. Pure and Applied Chem.*, **11**: 92–116.

Keston, A. S., 1956. Specific colorimetric enzymatic reagents for glucose. *Abstracts, A.C.S., Div. Biol. Chem.*, April: 31c.

Koike, H., 1954. Studies on carbohydrases of insects. I. Distribution of carbohydrases in several insects. *Zool. Mag. (Tokyo)*, **63**: 228–234.

Lasker, R., and A. C. Giese, 1956. Cellulose digestion by the silverfish *Ctenolepisma lineata*. *J. Exp. Biol.*, **33**: 542–553.

Lipke, H., and G. Fraenkel, 1956. Insect nutrition. *Ann. Rev. Entomol.*, **1**: 17–44.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.

Mahler, H. R., 1961. The use of amine buffers in studies with enzymes. *Ann. N. Y. Acad. Sci.*, **92**: 426–439.

Myrback, K., 1957. Inhibition of yeast invertase (saccharase) by metal ions. V. Inhibition by mercury compounds. *Arkiv. Kemi.*, **11**: 471–479.

Newcomer, W. S., 1952. The occurrence of beta-glucosidase in the digestive juice of *Porcellio* and *Armadillidium*. *Anat. Rec.*, **113**: 536.

Newcomer, W. S., 1954. The occurrence of β-glucosidase in the digestive juice of the cockroach, *Periplaneta americana* L. *J. Cell. Comp. Physiol.*, **43**: 79–86.

Newcomer, W. S., 1956. Digestive carbohydrases of the wood louse, *Porcellio*. *Physiol. Zoöl.*, **29**: 157–162.

Pearse, A. G. E., 1960. Histochemistry. Little, Brown and Co., Boston. 998 pp.

Peterson, E. A., and H. A. Sober, 1956. Chromatography of proteins. I. Cellulose ion exchange adsorbents. *J. Amer. Chem. Soc.*, **78**: 751–775.

Powning, R. F., and H. Irzykiewicz, 1962. β-glucosidase in the cockroach (*Periplaneta americana*) and in the puffball (*Lycoperdon perlatum*). *Comp. Biochem. Physiol.*, **7**: 103–115.

Reisfeld, R. A., V. J. Lewis and D. E. Williams, 1962. Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature*, **195**: 181–183.

Robinson, D., 1956. The fluorometric determination of β-glucosidase: its occurrence in the tissues of animals, including insects. *Biochem. J.*, **63**: 39–44.

Somogyi, M., 1952. Notes on sugar determination. *J. Biol. Chem.*, **195**: 19–23.

Stay, B., and L. M. Roth, 1962. The colleterial glands of cockroaches. *Ann. Entomol. Soc. Amer.*, **55**: 124–130.

Trevelyan, W. E., D. P. Procter and J. S. Harrison, 1950. Determination of sugars on paper chromatograms. *Nature*, **166**: 444–445.

Veibel, S., 1937–38. Über den Tolnol-Effekt bei der fermentativen Hydrolyse von β-d-glucosiden. *Enzymologia*, **2**: 367–372.

Veibel, S., 1951. *In:* J. B. Summer and K. Myrbäck (Editors), The Enzymes, Vol. I, Acad. Press, Inc., New York. pp. 583.

Weidenhagen, R., 1932. Spezifität und Wirkungmechanismus der Carbohydrasen. *Ergebn. Enzymforsch.*, **1**: 168–208.

White, J. W., Jr., and M. H. Subers, 1961. A glucose oxidase reagent for maltose assay. *Anal. Biochem.*, **2**: 380–384.